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Title: Hydrogen peroxide in exhaled air: A source of error, a paradox and its resolution

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Body: Background: The concentration of hydrogen peroxide (H₂O₂) in exhaled air has been reported to be elevated in asthma and COPD, but the data are inconsistent and difficult to reproduce. Notably, a relevant concentration of H₂O₂ can be found in ambient air. Therefore, we examined the association between H₂O₂ in ambient and exhaled air. Methods: Exhaled breath condensate (EBC) of 12 COPD patients and 9 healthy subjects was collected with an inhalation filter (F; efficiency 81%) or without (nF). Ambient air condensate (AAC) was collected in parallel and all samples were analysed for H₂O₂. Additionally, ambient H₂O₂ concentration was recorded by an analyser for atmospheric H₂O₂. Results: H₂O₂ concentration in AAC (3.60±1.40µM, mean±SD) was higher (p<0.01) than in EBC (Table). It showed meteorological variations concordant with atmospheric measurements. In both groups studied, the inhalation filter caused a reduction of H₂O₂ values (p<0.01). Despite the comparatively low levels in exhaled air, analysis by means of a mathematical model revealed an endogenous H₂O₂ contribution which was more pronounced when using the inhalation filter.

median (interquartile range), µM	COPD	control
exhaled F	0.42 (0.13)	0.45 (0.22)
exhaled nF	0.78 (0.51)	0.75 (0.32)
endog. (mucosa conc. equivalent)	0.66 (1.90)	0.69 (3.40)

Conclusion: The paradox of low H₂O₂ values in exhaled air assessed by EBC dissolves when taking into

account the reconditioning of inhaled air containing H_2O_2 . This may partially explain the heterogeneity of study results and their limited reproducibility. Still, there seems to be endogenous H_2O_2 production but its valid determination requires inhalation filters. This suggests a reanalysis of studies from the literature.